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Original Article

Uliginosin B, a natural phloroglucinol derivative with antidepressant-like activity, increases Na^+,K^+-ATPase activity in mice cerebral cortex

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ABSTRACT

Uliginosin B, a phloroglucinol isolated from *Hypericum polyanthemum* Klotzsch ex Reichardt, Hypericaceae, has antidepressant-like effect in the forced swimming test in rodents and inhibits monoamines neuronal reuptake without binding to their neuronal carriers. Studies showed the involvement of Na^+,K^+-ATPase brain activity in depressive disorders, as well as the dependence of neuronal monoamine transport from Na^+ gradient generated by Na^+,K^+-ATPase. This study aimed at evaluating the effect of uliginosin B on Na^+,K^+-ATPase activity in mice cerebral cortex and hippocampus (1 and 3 h after the last administration) as well as the influence of veratrine, a Na^+ channel opener, on the antidepressant-like effect of uliginosin B. Mice were treated (*p.o.*) with uliginosin B single (10 mg/kg) or repeated doses (10 mg/kg/day, 3 days). Acute administration reduced the immobility in the forced swimming test and tail suspension test and increased Na^+,K^+-ATPase activity in cerebral cortex 1 h after treating, whereas the repeated treatment induced the antidepressant-like effect and increased the Na^+,K^+-ATPase activity at both times evaluated. None treatment affected the hippocampus enzyme activity. Veratrine pretreatment prevented uliginosin B antidepressant-like effect in the forced swimming test, suggesting the involvement of Na^+ balance regulation on this effect. Altogether, these data indicate that uliginosin B reduces the monoamine uptake by altering Na^+ gradient.

Introduction

Based on the well-known efficacy of *Hypericum perforatum* (St. John's wort herb) for the treatment of mild to moderate depression (Linde, 2009) our group has been studying chemical and pharmacological features of South Brazilian *Hypericum* species (Daudt et al., 2000; Ferraz et al., 2002; Viana, 2007; Viana et al., 2003, 2005, 2006, 2008; Stein et al., 2012). *Hypericum polyanthemum* Klotzsch ex Reichardt, Hypericaceae, extracts have shown antinociceptive (Viana et al., 2003; Haas et al., 2010) and antidepressant-like effects in rats and mice (Stein et al., 2012). Its major chemical constituents are three benzopyrans, named HP1 (6-isobutyryl-5,7-dimethoxy-2,2-dimethyl-benzopyran), HP2 (hydroxy-6-isobutyryl-5-methoxy-2,2-dimethyl-benzopyran) and HP3 (5-hydroxy-6-isobutyryl-7-methoxy-2,2-dimethyl-benzopyran), and a phloroglucinol derivative, uliginosin B (Von Poser et al., 2006). Uliginosin B (**1**) has a dimeric structure consisting of phloroglucinol and filicinic acid moieties (Rocha et al., 1995; Nör et al., 2004; Duarte et al., 2014), and seems to be responsible for the antidepressant-like effects observed in animals behavioral tests (Stein et al., 2012). This compound showed antidepressant-like effect in the forced swimming test in mice, which was prevented by the impairment of the monoaminergic neurotransmission *in vivo*; it also inhibited the sinaptossomal uptake of dopamine, noradrenaline and serotonin, but interestingly it did not interact with their respective site on neuronal carriers (Stein et al., 2012). These findings suggest that

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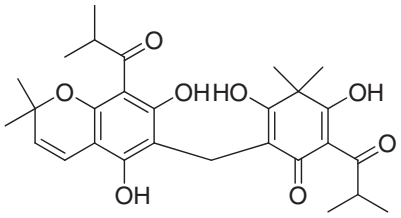
uliginosin B acts by a distinct mechanism than the classical antidepressant drugs, which act by blocking monoamine transporters.



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Monoamine transporters are located in the plasma membrane and are driven by the electrochemical gradient of Na^+ generated by Na^+,K^+-ATPase (Nelson and Lill, 1994). It has been shown that some antidepressants, such as amitriptyline, nortriptyline, imipramine and desipramine, inhibit the Na^+,K^+-ATPase activity (Carfagna and Muhoberac, 1993; Sanganahalli et al., 2000; Viola and Arnaiz, 2007). On the other hand, dopamine, noradrenaline and serotonin are able to stimulate the activity of this enzyme (Viola and Arnaiz, 2007).

Na^+,K^+-ATPase activity is essential to the brain normal function, since the flow of Na^+ and K^+ ions across the membrane is necessary to neuronal excitability, regulation of osmotic balance, cell volume and intracellular transport of molecules linked to the co-transport of Na^+, such as glucose, amino acids and neurotransmitters (Kaplan, 2002; Jorgensen et al., 2003). Several experimental studies have described the involvement of Na^+,K^+-ATPase brain activity in the depressive disorders (Zanatta et al., 2001; Gamaro et al., 2003; Vasconcellos et al., 2005; Acker et al., 2009). Mania and bipolar depression have been associated with increased intracellular Na^+ concentrations (Li and El-Mallakh, 2004). Rats exposed to chronic variable stress (CVS) developed despair-like endophenotypes and had decreased hippocampal and amygdalar Na^+,K^+-ATPase activity (Crema et al., 2010). The antidepressant fluoxetine and the mood stabilizer lithium simultaneously prevented despair induced by CVS and prevented the decrease in Na^+,K^+-ATPase activity (Vasconcellos et al., 2005).

The observation that several neuroactive drugs act on Na^+ channels also indicates the importance of Na^+ gradient in brain disorders neurobiology. Sodium channels are voltage-dependent transmembrane proteins responsible for the increase of permeability to sodium, which initiates and propagates the action potential in excitable cells (Cestèle and Catterall, 2000; Bourin et al., 2009). The Na^+ channels are targets to different classes of drugs such as anticonvulsants, local anesthetics and antiarrhythmics (Rasgdale et al., 1996). Some anticonvulsants (carbamazepine, lamotrigine, phenytoin, topiramate, valproate sodium) are also used to treat bipolar disorder (Reinares et al., 2012) and lamotrigine has been used specially to treat bipolar depression (Leng et al., 2013).

In this context, the aim of the present study was to evaluate the effect of the acute and sub-acute administration of uliginosin B on Na^+,K^+-ATPase activity in mice cerebral cortex and hippocampus and the involvement of sodium channels in uliginosin B antidepressant-like effect.

Materials and methods

Plant material

Hypericum polyanthemum Klotzsch ex Reichardt, Hypericaceae aerial parts were collected at Caçapava do Sul, in the state of Rio Grande do Sul – Brazil (October, 2008). The voucher specimens were deposited at the herbarium of the Federal University of Rio Grande do Sul (ICN Bordignon, 3118 Herbário do Departamento de Botânica – Instituto de Biociências – UFRGS). The plant collection was authorized by IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) (n° 003/2008; Protocol 02000.001717/2008 – 60).

Preparation of extract

H. polyanthemum lipophilic extract was obtained from dried and powdered plant material (300 g) extracted with cyclohexane (plant/solvent ratio 1:10, w/v) by static maceration for 48 h. The extract was evaporated to dryness under reduced pressure at 45 °C to eliminate the solvent, yielding a free solvent extract termed POL (3.5%). Then, the extract was treated with acetone to remove the waxes, according to Rocha et al. (1994), producing an insoluble residue, which was eliminated by filtering with paper filter.

Characterization of the extract by HPLC and uliginosin B isolation

The extract was dissolved in HPLC grade methanol (2 mg/ml), filtered (0.22 μm pore size, Merck) and analyzed by high performance liquid chromatography Water HPLC system (Milford, MA, USA). Uliginosin B (**1**) determination was carried out with an isocratic solvent condition (95% CH_3CN, 5% H_2O, 0.01% TFA) through a Waters Nova-Pack C18 column (4 μm, 3.9 mm × 150 mm) adapted to a guard column Waters Nova-Pack C18 60A (3.9 mm × 20 mm), flow rate of 1 ml min^{-1} and UV detection at 220 nm, according to the method previously described (Nunes et al., 2009). Uliginosin B concentration was determined as 16%, isolated by means of preparative thin layer and column chromatography and finally identified by ^1H–^{13}C NMR as described elsewhere (Rocha et al., 1995; Ferraz et al., 2002; Nör et al., 2004).

Animals

Behavioral and biochemical tests were carried out with male CF1 mice (25–30 g) purchased from Fundação Estadual de Produção e Pesquisa em Saúde, RS (Brazil). The animals were housed by five in plastic cages (17 cm × 28 cm × 13 cm) and were kept under a 12 h light/dark cycle (lights on at 7 a.m.) at constant temperature of 23 ± 1 °C with free access to standard certified rodent diet and tap water. All experimental protocols were approved by The Animal Care Local Ethical Committee (CEUA UFRGS; Protocol 18518), and performed according to Brazilian law (Brazil, 2008), which are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and International Guiding Principles for Biomedical Research Involving Animals (Bankowski, 1985).

Experimental design

Mice (n = 8 per group) were treated by gavage (10 ml/kg) with uliginosin B (**1**) acutely (10 mg/kg, *p.o.*) or sub-acutely (10 mg/kg, *p.o.*, once a day, during 3 days), based on previous results of our group (Viana et al., 2008). Control groups were performed for both treatment regimens and the animals were treated with vehicle (saline + polisorbate 80 2.0%). Different groups were used for biochemical and behavioral experiments (tail suspension test, TST – and forced swimming test, FST).

Time-course of uliginosin B antidepressant-like effect on the TST and FST was evaluated by testing independent groups of mice which were acutely or repeatedly treated with uliginosin B (10 mg/kg, *p.o.*) or vehicle. The animals were evaluated in the behavioral assays only once after treatment. Measurement of Na^+,K^+-ATPase activity was performed using animals that were not submitted to any behavioral test: different groups (acutely or

repeatedly treated) were euthanized by decapitation 1 or 3 h after receiving the last drug administration and brain structures were removed immediately.

In another set of experiments, we investigated the possible contribution of sodium channels to uliginosin B antidepressant-like effect. We evaluated the influence of the pre-treatment with veratrine, a Na^+ channel opener, on the FST. Initially, dose-response experiments were performed in the FST and locomotor activity in order to determine the dose of veratrine to be used: veratrine (0.06, 0.125 and 0.5 mg/kg, *i.p.*) was administered 45 min before the test. The sub effective dose was defined as the dose not able to reduce immobility in the FST and with no effect on locomotor activity (Centurião et al., 2014). The association of veratrine and uliginosin B consisted of the pretreatment with veratrine (0.06 mg/kg) or vehicle, *i.p.*, 45 min before the FST, plus uliginosin B or vehicle, *p.o.*, 30 min before the test.

Assays

Tissue preparation

Cerebral cortex and hippocampus were homogenized in 10 volumes (1:10, w/v) of 0.32 mM sucrose solution containing 5 mM HEPES and 1 mM EDTA, pH 7.5. The homogenates were centrifuged at $1000 \times g$ for 10 min and the supernatants were removed for Na^+,K^+-ATPase activity determination.

Na^+,K^+-ATPase activity assay

The reaction mixture for Na^+,K^+-ATPase assay contained 5 mM $MgCl_2$, 80 mM NaCl, 20 mM KCl, and 40 mM Tris–HCl, pH 7.4, in a final volume of 200 μl. After 10 min of pre-incubation at 37 °C, the reaction was initiated by the addition of ATP to a final concentration of 3 mM, and incubated for 20 min. Controls were carried out under the same conditions with the addition of 1 mM ouabain. Na^+,K^+-ATPase activity was calculated by the difference between the two assays according to the method described by Wyse et al. (2000). Released inorganic phosphate (Pi) was measured by the method of Cham et al. (1986). Specific activity of the enzyme was expressed as nmol Pi released per min per mg of protein.

Protein determination

Protein concentration was determined by the Bradford method (1976) using bovine serum albumin as standard.

Behavioral experiments

Tail suspension test (TST)

The TST was conducted according to Steru et al. (1985) with minor modifications (Müller et al., 2012). Mice were adapted to the laboratory conditions 1 h before the experiment. Animals were suspended by tail 60 cm above the floor using adhesive tape (1 cm from the tip of the end) in a dim light room. Immobility time was recorded (in seconds) by a blind to treatment observer during 6 min. Mice were considered immobile when they hung passively and completely motionless.

Forced swimming test (FST)

The FST was carried out according to Porsolt et al. (1978) with minor modifications standardized and validated in our laboratory (Viana et al., 2005). Mice were adapted to the laboratory conditions 1 h before being exposed to the FST. The animals were individually forced to swim in a cylinder pool (10 cm diameter, 13 cm high, water at 22 ± 1 °C) and the total time of immobility during 6 min was scored (in seconds). Immobility time was recorded when the mouse remained floating motionless or making only the movements necessary to keep its head above water.

Locomotor activity

The spontaneous locomotor activity was performed in the open-field (Viana et al., 2005). Forty-five minutes after the administration, mice were placed in a transparent acrylic box measuring 45 cm × 30 cm × 30 cm with a dark bottom divided into 24 equal quadrants. They were evaluated during 10 min, after 5 min habituation. The number of crossings was recorded by an observer blinded to treatments.

Statistical analysis

Data were expressed as mean + SEM of the mean. Data from biochemical analysis were analyzed by Student's *t* test. Behavioral experiments data were analyzed by one-way or two-way ANOVA followed by Student–Newman–Keuls. Differences were considered statistically significant at $p < 0.05$. The statistical procedures were performed using the Sigma Stat software 2.03 (Jandel Scientific Corporation).

Results

Acute administration of uliginosin B increased Na^+,K^+-ATPase activity in cerebral cortex 1 h after treatment [$t(9) = 2.447$, $p < 0.05$] (Fig. 1A), but not after 3 h [$t(8) = 0.323$, $p = 0.755$] (Fig. 1B). In the hippocampus, Na^+,K^+-ATPase activity was not altered at both times studied: 1 h [$t(9) = 0.898$, $p = 0.393$] (Fig. 1C) and 3 h [$t(8) = 1.155$, $p = 0.281$] (Fig. 1D).

Sub-acute administration of uliginosin B increased Na^+,K^+-ATPase activity in cerebral cortex 1 h after the last administration [$t(10) = 3.300$, $p < 0.01$] (Fig. 2A), as well as 3 h after [$t(9) = 2.518$, $p < 0.05$] (Fig. 2B). No alterations were verified in the hippocampus at the two different times: 1 h [$t(10) = 1.494$, $p = 0.166$] (Fig. 2C) and 3 h [$t(8) = 0.788$, $p = 0.449$] (Fig. 2D).

The acute administration of uliginosin B (10 mg/kg, *p.o.*) in the TST and FST are shown in Fig. 3. One-way ANOVA showed a significant effect of uliginosin B administration in the TST [$F(2,25) = 4.411$; $p < 0.05$]; *post hoc* analysis indicated a reduction on the immobility time at 1 h ($p < 0.05$), but not at 3 h after administration ($p = 0.079$) when compared to the control group (Fig. 3A). The same effect was observed when animals were submitted to the FST [$F(2,30) = 11.136$; $p < 0.001$]: *post hoc* analysis indicated a significant decrease in the immobility time 1 h after administration ($p = 0.076$), but not after 3 h ($p > 0.05$) (Fig. 3B).

The effect of sub-acute administration of uliginosin B (10 mg/kg/day, *p.o.*) is shown in Fig. 3C. One-way ANOVA revealed a significant effect of uliginosin B [$F(2,21) = 6.360$; $p < 0.01$] and *post hoc* analysis indicated a significant anti-immobility effect at 1 h ($p < 0.01$) and 3 h ($p < 0.05$) after the last administration when compared to control group.

The anti-immobility effect of uliginosin B (10 mg/kg, *p.o.*) was prevented by the pretreatment with veratrine (0.06 mg/kg, *i.p.*) [two-way ANOVA: $F_{\text{pre-treatment} \times \text{treatment}}(1,31) = 6{,}205$, $p = 0.019$] (Fig. 4). The co-administration of veratrine and uliginosin B has no effect when compared to the uliginosin B and vehicle (Tween), thus veratrine affect the antidepressant-like effect of uliginosin B in mouse FST.

Discussion

In the present study, we demonstrated that uliginosin B, the main phloroglucinol derivative from *H. polyanthemum*, increases activity of Na^+,K^+-ATPase in mice cerebral cortex, but not in hippocampus. This effect was treatment regimen dependent, being sustainable only in animals that were repeatedly treated. The same temporal activity profile was observed in the TST and

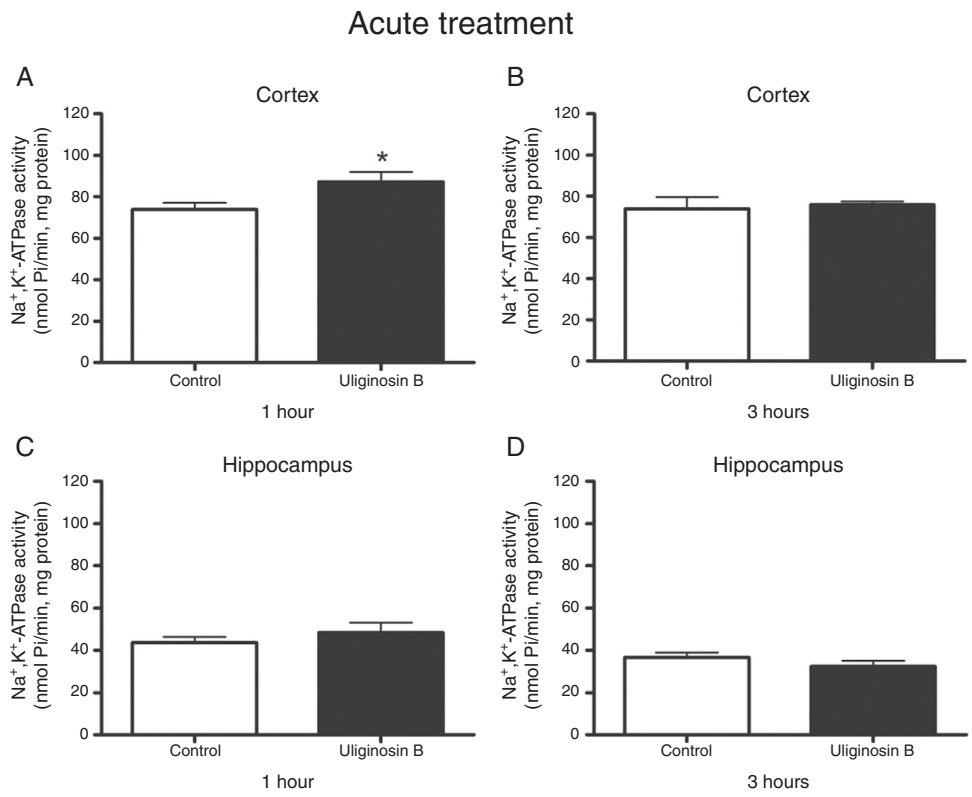

Fig. 1. Effect of the acute administration of uliginosin B on brain Na^+,K^+-ATPase activity. Mice were acutely treated with uliginosin B (10 mg/kg, *p.o.*) and euthanized by decapitation 1 or 3 h after the last administration; immediately cerebral cortex and hippocampus were removed to measure enzyme activity. Student *t* test; values are expressed as mean + SEM ($n = 5–6$). Difference from Control $^*p < 0.05$.

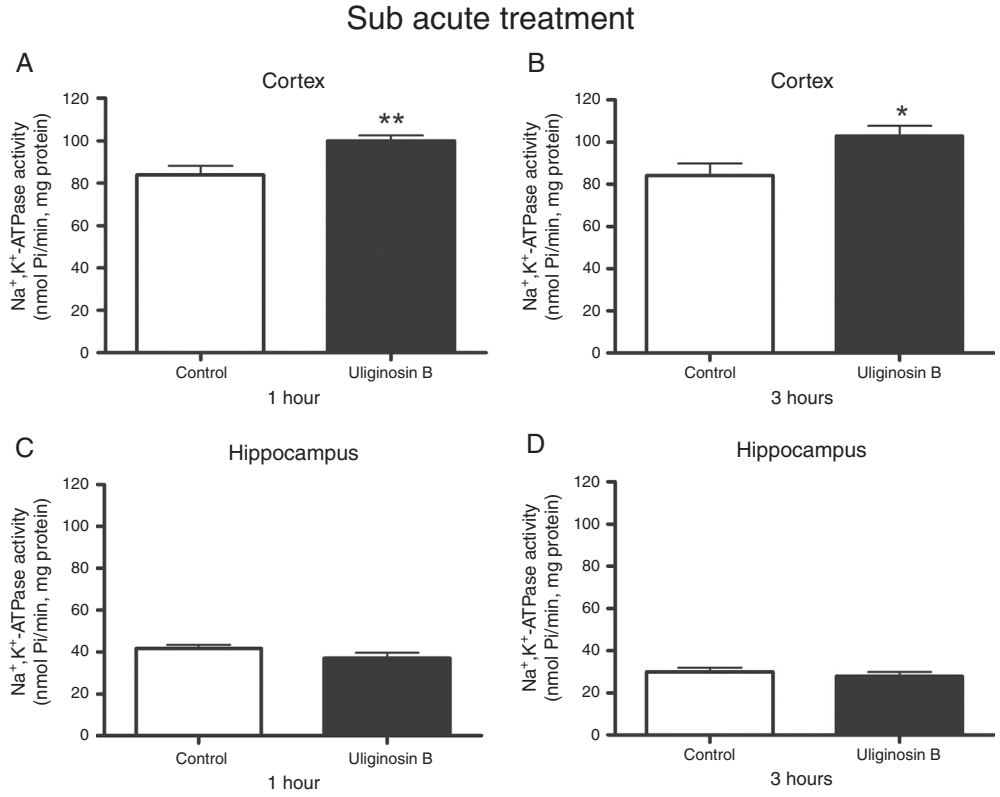

Fig. 2. Effect of the sub-acute treatment of uliginosin B on brain Na^+,K^+-ATPase activity. Mice were repeatedly treated with uliginosin B (10 mg/kg/day, 3 days, *p.o.*) and euthanized by decapitation 1 or 3 h after the last administration; immediately cerebral cortex and hippocampus were removed to measure enzyme activity. Student *t* test; values are expressed as mean + SEM ($n = 6$). Difference from Control $^*p < 0.05$.

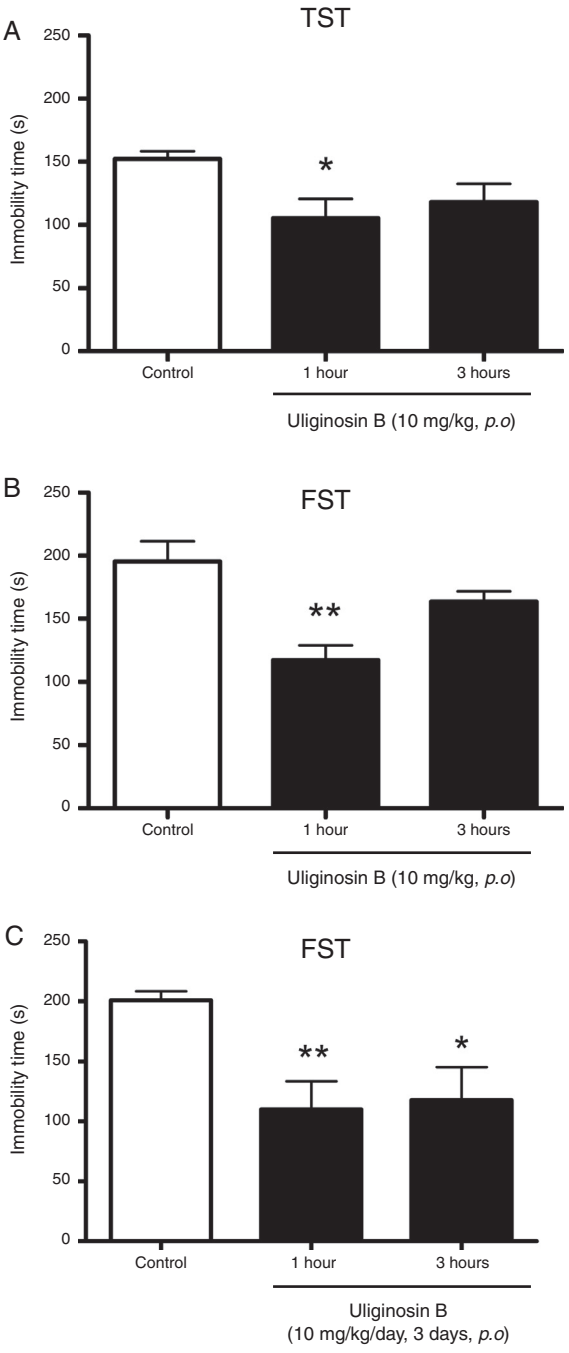

Fig. 3. Effect of the acute and sub-acute treatment of uliginosin B (10 mg/kg, *p.o.*) on the immobility time. Mice were acutely treated with uliginosin B and submitted to test, 1 or 3 h after administration: tail suspension test (TST, panel A) and forced swimming test (FST, panel B); mice were treated for 3 days, once a day and submitted to FST 1 or 3 h after administration (FST, panel C). One-way ANOVA followed by Student–Newman–Keuls; values are expressed as mean + SEM (n = 8–10). Difference from Control $^{*}p < 0.05$, $^{**}p < 0.01$.

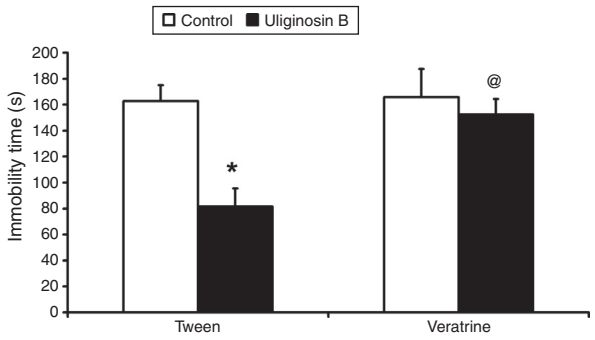

Fig. 4. Effect of the pretreatment of mice with veratrine (0.06, mg/kg *i.p.*) on the anti-immobility effect of uliginosin B (10 mg/kg, *p.o.*) in the FST. Independent groups of mice were pretreated (*i.p.*) with vehicle (saline + polisorbate 80 2% = tween) and treated (*p.o.*) with vehicle (Tween – Control group) or uliginosin B (Tween – Uliginosin B group); or pretreated (*i.p.*) with veratrine and treated (*p.o.*) with vehicle (Veratrine – Control group) or uliginosin B (Veratrine – Uliginosin B group). Values expressed as mean + SEM (n = 8–10). Two-way ANOVA followed by Student–Newman–Keuls. * Difference from Tween – Uliginosin B *versus* Tween – Control, $p < 0.05$. @Difference from Tween – Uliginosin B *versus* Veratrine – Uliginosin B, $p < 0.05$.

FST suggesting that the uliginosin B effect is, at least in part, due to its action on Na^+,K^+-ATPase. Furthermore, the pretreatment with veratrine, a Na^+ channel opener, prevented the uliginosin B antidepressant-like effect, reinforcing that the activity of uliginosin B involves the regulation of Na^+ balance.

Depression is defined clinically as a pathological complex of psychological, neuroendocrine and somatic symptoms that cannot be reproduced in animals. However, in mice, specific measurable behaviors' can be assayed such as FST and TST which are a good screening tools with good reliability and predictive validity (Petit-Demouliere et al., 2005; Cryan et al., 2005; Castagné et al., 2009). The effect of uliginosin B in reducing immobility time in the TST and FST reinforces our previous results that have already demonstrated the antidepressant-like effect of this phloroglucinol derivative (Stein et al., 2012). In addition, in this study we moved on the possible mode of action of uliginosin B by studying its effect on the enzyme Na^+,K^+-ATPase. Clinical (Goldstein et al., 2006, 2009; Tochigi et al., 2008) and preclinical studies (Acker et al., 2009; Gamaro et al., 2003; Vasconcellos et al., 2005; Crema et al., 2010; Kirshenbaum et al., 2011) reported that Na^+,K^+-ATPase is diminished in depressive disorders. Gamaro et al. (2003) demonstrated that the Na^+,K^+-ATPase activity decreased in rats hippocampus subjected to chronic stress model of depression, effect that was reversed by the repeated treatment with fluoxetine and lithium (Vasconcellos et al., 2005), whereas tricyclic antidepressants inhibited the enzyme activity (Sanganahalli et al., 2000).

Although the animals were not subjected to any depression model, the results showed that uliginosin B increased Na^+,K^+-ATPase activity in cerebral cortex about 18% 1 h after the last administration, but it did not alter the enzyme activity after 3 h. In the sub-acute treatment, uliginosin B was able to increase in 20% the enzyme activity 1 and 3 h after the last administration, suggesting a longstanding effect of the repeated treatment (for 3 days). These findings are consistent with Zanatta et al. (2001), who demonstrated that the chronic administration of fluoxetine (14 days) increases the enzyme activity, fact that can contribute to fluoxetine therapeutic efficacy.

On the other hand, none of the uliginosin B treatment regimens (acute and sub-acute) have changed the enzyme activity in the hippocampus. Morphological and neurochemical alterations have been reported in the hippocampus of depressed patients (Sheline et al., 2003). Thus, the lack of the effect of uliginosin B on the Na^+,K^+-ATPase activity in mice hippocampus may be due the fact that the animals were not submitted to any experimental model of chronic depression. This finding also suggests a selective effect of uliginison B on cortical Na^+,K^+-ATPase, which could be particularly relevant, since this brain structure is involved in depression neurobiology (Price and Drevets, 2010) and *post mortem* studies have demonstrated alterations on Na^+,K^+-ATPase activity in the cortex of depressed patients (Goldstein et al., 2006). Noteworthy, in a previous study our group has found similar results (Müller et al., 2015), where the Na^+,K^+-ATPase activity was increased in

the cortex, but not in the hippocampus, of animals treated with valepotriates. We speculate that this profile could be related to the irregular distribution of Na^+,K^+-ATPase isoenzymes. Three α isoforms are abundant in the brain: α1, expressed by neurons and glia; α2, predominant in glia; and α3, neuronal (McGrail et al., 1991). Tochigi et al. (2008) studied the gene expression pattern in postmortem brains of subjects with major depression and found that Na^+,K^+-ATPase α3 gene expression is decreased in prefrontal cortex of subjects with major and bipolar depression. Another study with Na^+,K^+-ATPase α3 heterozygous mice showed a reduction of 15% in neuronal Na^+,K^+-ATPase activity. We speculate that this profile could be related to the irregular distribution of Na^+,K^+-ATPase isoenzymes. Three α isoforms are abundant in the brain: α1, expressed by neurons and glia; α2, predominant in glia; and α3, neuronal (McGrail et al., 1991). Tochigi et al. (2008) studied the gene expression pattern in postmortem brains of subjects with major depression and found that Na^+,K^+-ATPase α3 gene expression is decreased in prefrontal cortex of subjects with major and bipolar depression. Another study with Na^+,K^+-ATPase α3 heterozygous mice showed a reduction of 15% in neuronal Na^+,K^+-ATPase activity. Furthermore, these animals were vulnerable to develop increased depression-like endophenotypes in a chronic variable stress model (Kirshenbaum et al., 2011). These data suggest that Na^+,K^+-ATPase α3 in cerebral cortex could be a target to new antidepressant drugs and to study the pathophysiology of depressive disorders.

The pre-treatment with veratrine prevented the anti-immobility effect of uliginosin B in the FST, effect that is in line with the literature, which demonstrated a similar profile to hyperbrasilol B (Centurião et al., 2014), hyperforin (Codagnone et al., 2007) and lamotrigine (Calabrese et al., 2008; Prica et al., 2008; Bourin et al., 2009). A recent study from our group showed that hyperbrasilol B, a natural dimeric phloroglucinol derivative from *H. caprifoliatum*, also had its anti-immobility effect prevented by veratrine, and it was able to increase Na^+,K^+-ATPase activity in mice hippocampus, but not cerebral cortex (Centurião et al., 2014). Hyperforin, a phloroglucinol derivative from *H. perforatum*, which is an European species worldwide used for depression, seems to exert its antidepressant action by mechanisms dependent on Na^+ channels, without altering the activity of Na^+,K^+-ATPase (Chatterjee et al., 1998a,b). In addition, hyperforin inhibits the reuptake of monoamines without binding to their transporters (Wonnemann et al., 2000). Several authors demonstrated the presence of a carrier-mediated monoamine transport mechanism, responsible for the entrance of dopamine, norepinephrine and serotonin in the nerve terminal, accompanied by Na^+ ions (Xhaard et al., 2008; Kristensen et al., 2011) mechanism abolished in the absence of Na^+ (Krueger, 1990; Gu et al., 1994; Pifl et al., 1997). Lamotrigine, in turn, is an anti-epiletic agent used at bipolar depression to maintenance treatment. It acts by stabilizing the presynaptic membrane through the blockade of voltage-gated Na^+ channels (Codagnone et al., 2007). Vitezic et al. (2008) demonstrated a partial protective effect of lamotrigine on the inhibition of Na^+,K^+-ATPase activity induced by kainic acid in rats prefrontal cortex and hippocampus. Also, Southam et al. (1998) demonstrated *in vitro* that lamotrigine inhibits the reuptake of serotonin, norepinephrine and dopamine, reinforcing the involvement of sodium gradient (Xhaard et al., 2008).

Considering the effect of uliginosin B on the activity of Na^+,K^+-ATPase and on Na^+ channels together with previous studies of our group demonstrating that uliginosin B inhibits the reuptake of monoamines in a different manner from most antidepressants (Stein et al., 2012) we can speculate that uliginosin B reduces the monoamine uptake by altering Na^+ gradient.

In conclusion, the present study represents one step ahead in the elucidation of the mechanism of action of the antidepressant-like activity of uliginosin B, a natural phloroglucinol derivative, suggesting that involvement of uliginosin B in regulation of Na^+ balance may occur through increased of Na^+,K^+-ATPase activity. The regulatory mechanism involving Na^+ may be regarded an important property for antidepressant-like activity of this phloroglucinol derivative.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the all experimental protocols were approved by The Animal Care Local Ethical Committee (CEUA UFRGS; Protocol 18518), and performed according to Brazilian law (Brazil, 2008), which are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and International Guiding Principles for Biomedical Research Involving Animals (Bankowski, 1985). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

ACS and LGM contributed in running the laboratory work, chromatographic analysis, analysis of the data and drafted the paper. AGKF, AB, AHB, FBC, EBS, JK, contributed to biological studies. GLVP contributed in collecting plant sample and identification, chemical analyses and critical reading of the manuscript. ATSW and SMKR planned the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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